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(54) Title: OSTEOINDUCTIVE PHARMACEUTICAL FORMULATIONS

(57) Abstract

Osteoinductive pharmaceutical formulations comprising antifibrinolytic agents such as epsilon amino acid caproic acid or other lysine analogues or serine protease inhibitors and cartilage and/or bone inductive proteins are disclosed. These formulations are useful in the treatment of cartilage and/or bone defects.

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OSTEOINDUCTIVE PHARMACEUTICAL FORMULATIONS

This application is a continuation-in-part of U.S. Serial No. 539,756 filed June 18, 1990.

This invention relates to cartilage and/or bone inductive pharmaceutical formulations. More specifically, it relates to the use of epsilon aminocaproic acid (EACA) or other lysine analogues, serine protease inhibitors or other antifibrinolytic agents in cartilage and/or bone inductive formulations.

Formulations of the invention comprise EACA or other lysine analogues, serine protease inhibitors or antifibrinolytic agents in conjunction with cartilage and/or bone inductive proteins such as BMP-2 (having been designated in the past as BMP-2A or BMP-2 Class I), BMP-3, or BMP-4 (having been designated in the past as BMP-2B and BMP-2 Class II) disclosed in PCT International Publications WO88/00205 and WO89/10409. Further cartilage and/or bone inductive proteins for use in the invention include BMP-5, BMP-6, and BMP-7 disclosed in PCT International Publication WO90/11366. BMP-8 disclosed in U.S. Serial No. 07/525,357 filed May 16, 1990 and 07/641,204 filed January 15,1991 is another cartilage and/or bone inductive protein for use in formulations of the invention.

In further embodiments the formulations may further comprise growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factors (TGF-α and TGF-β), and platelet derived growth factor (PDGF).

The formulations may also include an appropriate matrix, for instance, for sequestering and/or support of the composition and/or providing a surface for bone and/or cartilage formation. The matrix may provide sequestering of the cartilage and/or osteoinductive protein(s) and/or the

appropriate environment for presentation of the protein(s).

EACA is the presently preferred lysine analogue for use in formulations of the invention. The formulation may comprise other lysine analogues including trans-p-aminomethyl-cyclohexanecarboxylic acid (AMCA; tranexamic acid) (Amstat). The presence of EACA in osteoinductive formulations accelerates the amount of bone formation and/or decreases the amount of cartilage and/or bone inductive protein required.

EACA is known to have a fibrin stabilizing effect. It inactivates plasmin which is a serine protease. [Nilsson et al Lancet,1: 1233-1236 (1960)]. EACA has been shown to enhance new collagen synthesis in animals through blockage of the fibrinolytic system. [Brandstedt et al, Eur. Surg. Res. 12: 12-21 (1980)]. We have found that EACA increases the sensitivity of osteoinductive assays by accelerating the amount of cartilage and/or bone formed and/or decreasing the amount of cartilage/bone inductive protein required.

The invention further features a method for formulating the compositions of the invention, as well as use of the formulations in methods for treating a number of bone and/or cartilage defects, and periodontal disease. The formulations may also be employed in methods for treating various types of wounds and in tissue repair. These methods, according to the invention, entail administering a composition of the invention to a patient needing such bone and/or cartilage formation, wound healing or tissue repair. The method therefore involves administration of a therapeutically effective amount of a lysine analogue, serine protease inhibitor, or other antifibrinolytic agent and a therapeutically effective amount of a cartilage and/or bone inductive protein in a pharmaceutically acceptable carrier. These proteins include, for instance at least one of the "BMP" proteins disclosed in the co-owned applications described above.

In addition, these methods may further entail administration of other growth factors including EGF, FGF,

TGF-α, TGF-β, and PDGF.

Other aspects and advantages of the invention will be apparent based upon consideration of the following detailed description and preferred embodiments thereof.

Detailed Description of the Invention

The osteoinductive formulations of the invention comprise EACA, a lysine analogue, in conjunction with a cartilage and/or bone inductive factor in a pharmaceutically acceptable vehicle. Other synthetic lysine analogues which may be used in practice of the invention include trans-p-aminomethyl-cyclohexanecarboxylic acid (AMCA; tranexamic acid). Inhibitors of fibrin clot lysis, other than the lysine analogues mentioned above, for instance serine protease inhibitors, in conjunction with a cartilage and/or bone inductive factor may also comprise formulations of the invention. Such serine protease inhibitors may include aprotinin, α_2 antiplasmin and α_2 macroglobulin. A further fibrinolytic agent which may be useful in formulations of the invention is p-amino methyl benzoic acid.

The cartilage and/or bone inductive factors which may be used in formulations of the invention include, but are not limited to BMP-2, BMP-3 and BMP-4 disclosed in International Publications WO88/00205 and WO89/10409. Further cartilage and/or bone inductive proteins for use in the invention include BMP-5, BMP-6, and BMP-7 disclosed in PCT International Publication WO90/11366. BMP-8 disclosed in U.S. Serial No. 07/525,357 filed May 16, 1990 and 07/641,204 filed January 15,1991 is another cartilage and/or bone inductive protein for use in formulations of the invention.

The use of EACA in osteoinductive formulations accelerates the amount of bone formation and/or decreases the amount of cartilage and/or bone inductive protein required. For example, with EACA present significantly more bone formation is seen at seven days post implantation using the same amount of bone inductive factor as compared to

formulations without EACA.

In addition to formulations comprising EACA and cartilage/bone protein, formulations of the invention further comprise autologous blood. At the time of surgery the matrix (described below) is mixed with a sufficient quantity of the patients' blood, EACA and cartilage/bone protein. The suitability of autologous blood is based on its biocompatability and ready availability. Autologous blood may also be utilized instead of another matrix. Further formulations of the invention comprise EACA (or other fibrinolytic agents) autologous blood and cartilage/bone inductive protein.

In addition to the cartilage/bone protein, the formulations may include at least one other therapeutically useful agent including growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factors (TGF-α and TGF-β), and platelet derived growth factor (PDGF).

The formulations may also include an appropriate matrix, for instance, for delivery and/or support of the composition and/or providing a surface for bone and/or cartilage formation. The matrix may provide sequestering of the BMP protein or other cartilage/bone protein or other factors of the formulation and/or the appropriate environment for presentation of the formulation of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the cartilage and/or bone inductive proteins to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatability, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular

application of the formulations of the invention will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, poly(lactic acid), poly(glycolic acid) and polyanhydrides as well as coral. Other potential materials are biodegradable and biologically well defined, such as bone, tendon or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as poly(lactic acid) and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

The formulations of the invention may be employed in methods for treating a number of bone and/or cartilage defects, and periodontal disease. They may also be employed in methods for treating various types of wounds and in tissue repair. These methods, according to the invention, entail administering a composition of the invention to a patient needing such bone and/or cartilage formation, wound healing or tissue repair. The method therefore involves administration of a lysine analogue or other antifibrinolytic agent and a therapeutically effective amount of a cartilage and/or bone inductive protein in a pharmaceutically acceptable vehicle. These methods may further include the administration of the EACA and cartilage and/or bone inductive protein in conjunction with other growth factors including EGF, FGF, TGF-α, TGF-β, and PDGF.

A formulation of the present invention, which induces cartilage and/or bone formation in circumstances where bone and/or cartilage is not normally formed, has application in

the healing of bone fractures and cartilage defects in humans and other animals. A preparation employing lysine analogues such as EACA, serine protease inhibitors or other antifibrinolytic agents and a cartilage and/or bone inductive protein may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery. Formulations of the invention may be used in the treatment of periodontal disease, and in other tooth repair processes. Such formulations may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells.

The formulations of the invention may also be used in wound healing and related tissue repair. The types of wounds include, but are not limited to burns, incisions and ulcers. See, e.g. PCT Publication WO84/01106 for discussion of wound healing and related tissue repair.

The preparation of such physiologically acceptable formulations, having due regard to pH, isotonicity, stability and the like, is within the skill of the art. The therapeutic formulations are also presently valuable for veterinary applications due to the apparent lack of species specificity in cartilage and bone growth factor proteins. Domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with the proteins of the present invention.

The therapeutic method includes administering the formulation topically, systemically, or locally as an implant or device. When administered, the therapeutic formulation for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the formulation may desirably be encapsulated or injected in a viscous form for

delivery to the site of cartilage and/or bone or tissue damage. Topical administration may be suitable for wound healing and tissue repair.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of the formulation of the invention. Factors which may modify the action of the formulation include the amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the size of a wound, type of damaged tissue, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the type or types of bone and/or cartilage proteins present in the composition. The addition of other known growth factors, such as EGF, PDGF, TGF-α, TGF-β, and IGF-I and IGF-II to the final composition, may also effect the dosage.

The concentration of EACA utilized in the sheep experiments described below is 10^{-3}M. EACA is expected to have a wide margin of safety when used locally because up to 30 grams per 24 hours can be administered systemically without toxicity. [Hemostasis and Thrombosis Basic Principles and Clinical Practice pp. 380-384 (1988); Stefanini et al, J. of Urology 143: 559-561 (1990)].

Progress can be monitored by periodic assessment of cartilage and/or bone growth and/or repair. The progress can be monitored, for example, using x-rays, histomorphometric determinations and tetracycline labeling.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

EXAMPLE I

Rosen Modified Sampath-Reddi Assay

A modified version of the rat bone formation assay

described in Sampath and Reddi, Proc. Natl. Acad. Sci. U.S.A., 80:6591-6595 (1983) is used to evaluate bone and/or cartilage activity of a formulation of the invention comprising EACA and cartilage and/or bone inductive proteins. This modified assay is herein called the Rosen Assay. The ethanol precipitation step of the Sampath-Reddi procedure is replaced by dialyzing (if the composition is a solution) or diafiltering (if the composition is a suspension) the fraction to be assayed against water. EACA (Amicar) is dissolved in water and added to 20 mg rat matrix wetted with 0.1% TFA and bone inductive protein. The controls include samples containing the bone inductive protein without the EACA. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male Long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., Proc. Natl Acad Sci., 69:1601 (1972)].

The other half of each implant is fixed and processed for histological analysis. Glycolmethacrylate sections (1μm) are stained with Von Kossa and acid fuschin or toluidine blue to score the amount of induced bone and cartilage formation present in each implant. The terms +1 through +5 represent the area of each histological section of an implant occupied by new bone and/or cartilage cells and newly formed bone and matrix. Two scoring methods are herein described. In the first scoring method a score of +5 indicates that greater than 50% of the implant is new bone and/or cartilage produced as a direct result of protein in the implant. A score of +4, +3, +2 and +1 would indicate that greater than 40%, 30%, 20% and 10% respectively of the implant contains new cartilage and/or bone. The second scoring method (which hereinafter may be referred to as the modified scoring method) is as follows: three non-adjacent sections are evaluated from each implant

and averaged. "+/-" indicates tentative identification of cartilage or bone; "+1" indicates >10% of each section being new cartilage or bone; "+2", >25%; "+3", >50%; "+4", ~75%; "+5", >80%. The scores of the individual implants are tabulated to indicate assay variability.

As with other cartilage and/or bone inductive proteins such as the above-mentioned "BMP" proteins, the bone and/or cartilage formed is expected to be physically confined to the space occupied by the matrix. The implants containing rat matrix to which specific amounts of "BMP" protein or EACA and "BMP" protein have been added are removed from rats after approximately seven days and processed for histological evaluation. Representative sections from each implant are stained for the presence of new bone mineral with von Kossa and acid fuschin, and for the presence of cartilage-specific matrix formation using toluidine blue. The types of cells present within the section, as well as the extent to which these cells display phenotype are evaluated and scored.

EXAMPLE II

Biological Activity of EACA and BMP-2 in the Rosen Assay

The efficacy of formulations of the invention comprising EACA and BMP-2 is tested in the Rosen Assay described above. The experiments include BMP-2, EACA and autologous blood, as well as BMP-2 and autologous blood without EACA. Negative controls without rat matrix include BMP-2 alone; BMP-2 and autologous blood and BMP-2 with autologous blood and EACA. EACA is dissolved in water and added to the rat matrix wetted within 0.1% TFA and BMP-2 [described in International Publication WO88/00205; PNAS, (USA) 87:2220-2224 (March 1990)]. Amounts of EACA ranging from 13 μg to 6.9 mg are added per 20 mg of rat demineralized, guanidinium chloride extracted bone or from 1 mM to 0.5 M based on a volume of 100 μg per implant. Samples are frozen and lyophilized and implanted in rats for seven days. The modified scoring method

is utilized. In experiments containing autologous blood BMP-2 is mixed with the EACA and lyophilized in a siliconized glass tube. 100-200 ul of autologous blood is removed from the rat by orbital puncture and then added to the tube. The blood is allowed to clot for 1-2 hours; when firm, the clot is removed and implanted subcutaneously.

The amount of bone and/or cartilage formed increases with the use of EACA compared to samples lacking EACA when the same amount of cartilage/bone protein is utilized. The control samples do not result in any bone and/or cartilage formation. More specifically, in the presence of rat matrix, EACA increases the amount of bone formed as compared to BMP-2 without EACA at a moderate dose. EACA increases the amount of cartilage seen at a very low dose. Higher amounts of BMP-2 result in an increase of the amount of cartilage and bone (summed) formed and a more rapid appearance of bone.

Blood as a matrix dramatically increases the sensitivity of BMP-2 as compared to BMP-2 in the absence of blood. The recovery of any implant at all is quite small, in the absence of blood. The omission of EACA in these implants results in a lower recovery of activity and implants.

EXAMPLE III

Biological Activity of EACA and BMP-2 in a Sheep Model

An osteoperiosteal defect is created by excising a 2.5 cm midshaft segment from the right femur of skeletally mature sheep. Marrow contents and periosteum are removed from the exposed ends and the 2.5 cm gap stabilized with an anteriolateral metal fixation plate. Different materials are used to fill the gap in each of four groups of animals: (1) autologous bone graft from the cortical bone and iliac crest; (2) no implant ; (3) recombinant human BMP-2, described above, mixed with inactive bone matrix comprising ground sheep bone demineralized and extracted with guanidinium chloride and sterilized, plus EACA ; and (4) inactive bone matrix and EACA.

The highly purified human BMP-2, expressed in mammalian cells, reconstituted with inactive bone matrix is frozen and lyophilized. Following lyophilization blood is added and EACA added to a final concentration of 1 mM.

Femoral radiographs are performed weekly. Animals are sacrificed at 12 weeks post-op, and biomechanical testing [four-point bending to failure] followed by histological analysis, is performed on the limbs.

The untreated defect [group (2)] and the defect treated with inactive matrix [group (4)] failed to show radiographic healing by week 12. All defects treated with BMP-2 [group (3)] showed radiographic evidence of new bone formation beginning at week 5 and progressing to union by week 12. Defects treated with autologous graft [group (1)] also show union by week 12. The radiographic findings were confirmed by gross analysis: specimens from groups (2) and (4) examined at 12 weeks showed gross motion at a fibrous tissue seam across the segmental defect site, while the autologous graft and the BMP-2 treated sites were rigid. Biomechanical testing supported these results. At week 12, the average bending strength [expressed as a percentage of the contralateral intact femur] was 111% for autologous graft, 16% for groups (2) & (4), and 91% for defects treated with BMP-2. Histologic analysis of a defect treated with BMP-2 showed evidence of new endochondral bone formation at two weeks post-op.

EXAMPLE IV

Rat Orthotopic Model

A 5 mm osteoperiosteal segmental defect (2x diaphyseal diameter) is created in the mid shaft of the femur of 325-350 gm Sprague-Dawley rats. Internal fixation is achieved with a four hole polyethylene plate fixed with 0.062 mm threaded Kirschner wires. Marrow is flushed from the intramedullary cavity at each side of the osteotomy. Two separate experiments using the rat orthotopic model are

described below.

A. Three groups of fifteen rats are studied as follows:

Group I: A rat matrix is implanted into the defect as a control.

Group II: A rat matrix, EACA and 1 μg microgm of BMP-2 are implanted as the low test dose.

Group III: A rat matrix, EACA and 8 μg microgm of BMP-2 are implanted as the high test dose.

EACA is added to 1 mM final concentration (assuming a volume of 50 ul) at the same time as the BMP-2 to the rat demineralized, guanidinium chloride-extracted bone. The sample is frozen and lyophilized. All rats are evaluated on day 7 for angiogenesis effect using dynamic quantitative bone scanning via intracardiac injection. The ratio of the total counts recorded over 60 seconds of the operated femur to the normal femur was then determined for each rat.

Bone formation in all rats is evaluated with serial radiographs taken at 1,2,3,4,5,6 and 9 weeks. The area of the defect occupied by bone is estimated by planimetry on lateral radiographs and recorded for each rat (as a percent of total defect area).

One rat is sacrificed each week for histologic analysis. Tissue from the grafted area and its surrounding bone is excised, decalcified, sectioned and stained with hematoxylin and eosin. Histological findings are recorded for sections taken parallel to the longitudinal axis of the bone extending over the entire length of the defect.

Those rats in which union occurrs across the defect are subjected to mechanical torsion testing to failure to determine the maximum torque, angular displacement, energy absorption and stiffness of the operated femur with the results compared to the contralateral normal femur.

B. Four groups of rats are studied as follows:

Group I: BMP-2 and autologous blood are implanted into the defect.

Group II: BMP-2, autologous blood and EACA are implanted into the defect.

Group III: A PLGA matrix, BMP-2 and autologous blood are implanted in the defect.

Group IV: A PLGA matrix, BMP-2, autologous blood and EACA are implanted in the defect.

The amount of BMP-2 is 15μg, 5μg and 1.5μg with 0.5M Arg in imidazole buffer pH 6.5. The amount of EACA per implant is 10mM. The BMP-2 and EACA are added to 56μl autologous blood. After mixing, the blood solution is added to the PLGA particles (80μl, ca.24mg.) described below and thoroughly mixed. After 90-120 minutes the clot is implanted in the rat femur gap.

The porous PLGA particles [a 50:50 (molar) random copolymer of lactic acid and glycolic acid (PLGA) having an average molecular weight of 30-40K, a number average molecular weight of about 20K (by GPC relative to polystyrene standards), and an inherent viscosity of 0.35-0.45 dL/g] are prepared by a solvent evaporation technique. PLGA is dissolved in CH_2Cl_2 (15% w/v), and porosigen is suspended in this solution. The resulting solution is added to an excess poly(vinyl alcohol) aqueous solution (0.1% w/v). After a few hours of stirring, the particles are hardened in excess cold ethanol (95%). The resulting particles are washed with WFI and lyophilized to give a free-flowing product.

The rats are sacrificed after nine weeks. Ex-vivo analysis of the new bone is performed radiographically relative to the contralateral femur. Preliminary results taken after three weeks indicate that the presence of EACA at marginal (5μg) and low (1.5μg) doses of BMP-2 appears to

increase the rate of unions observed as compared to the controls without EACA. Preliminary data indicates a lesser effect of the EACA at higher doses (15μg) of BMP-2 than with the lower doses. EACA particularly appears to increase the response in the BMP-2/autologous blood implants.

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are believed to be encompassed within the claims appended hereto.

What is claimed is:

1. A pharmaceutical formulation comprising an antifibrinolytic agent and a therapeutically effective amount of a cartilage and/or bone inductive factor in a pharmaceutically acceptable vehicle.

2. The formulation of claim 1 wherein said antifibrinolytic agent is selected from the group consisting of lysine analogues and serine protease inhibitors.

3. A pharmaceutical formulation comprising epsilon amino caproic acid and a therapeutically effective amount of a cartilage and/or bone inductive factor in a pharmaceutically acceptable vehicle.

4. The composition of claim 1 wherein said cartilage and/or bone inductive is selected from the group consisting of BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7 and BMP-8.

5. The formulation of claim 1 further comprising autologous blood.

6. The formulation of claim 3 further comprising autologous blood.

7. The formulation of claim 1 further comprising a pharmaceutically acceptable matrix.

8. The formulation of claim 1 further comprising a growth factor selected from the group consisting of IGF-I, IGF-II, PDGF, FGF, EGF, TGF-α and TGF-β.

9. A pharmaceutical formulation comprising BMP-2, epsilon amino caproic acid, autologous blood and a PLGA matrix.

10. A method of formulating an osteoinductive preparation by combining epsilon amino caproic acid with at least one osteoinductive factor.

11. The method of claim 10 wherein said osteoinductive factor is selected from the group consisting of BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, BMP-7 and BMP-8.

12. A method for treating cartilage and/or bone defects comprising administering a formulation comprising a lysine analogue and a therapeutically effective amount of a cartilage and/or bone inductive factor in a pharmaceutically acceptable vehicle.

13. The method of claim 12 wherein said lysine analogue is epsilon amino caproic acid.

14. A pharmaceutical formulation of claim 2 wherein said serine protease inhibitor is selected from the group consisting of aprotinin, α_2 antiplasmin and α_2 macroglobulin.

15. A pharmaceutical formulation comprising autologous blood and a therapeutically effective amount of a cartilage and/or bone inductive factor in a pharmaceutically acceptable vehicle.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/04337

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 37/02 //(A 61 K 37/02 A 61 K 31:195)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	Chemical Abstracts, vol. 75, no. 7, 16 August 1971, (Columbus, Ohio, US), A.G. Korobkina et al.: "Action of epsilon-aminocaproic acid during combined radiation injuries", see page 60, abstract 45404a, & Voenno-Med.Zh. 1971, (2), 42-6 ---	1-11,14 ,15
P,A	EP,A,0430200 (BEHRINGWERKE AG) 5 June 1991 -----	1-11,14 ,15

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
02-10-1991	30. 10. 91
International Searching Authority EUROPEAN PATENT OFFICE	**Signature of Authorized Officer** Dagmar Frank

Mme Dagmar FRANK

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers 12 and 13 because they relate to subject matter not required to be searched by this Authority, namely:

Please see PCT-RULE 39.1 (iv):
Methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

2. [ ] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9104337 SA 49025

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 23/10/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0430200	05-06-91	DE-A- 3939346	06-06-91
		AU-A- 6702990	06-06-91
		JP-A- 3170437	24-07-91